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Molecular Cloning and Differential Expression of the Maize Ferredoxin Gene Family[1]

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ABSTRACT

In maize (*Zea mays* L.), four ferredoxin (Fd) isoproteins, Fd I to Fd IV, are differentially distributed in photosynthetic and nonphotosynthetic organs of young seedlings (Y Kimata, T Hase [1989] Plant Physiol 89: 1193–1197). To understand structural characteristics of the Fd isoproteins and molecular mechanism of the differential expression of their genes, we have cloned and characterized three different maize Fd cDNAs. DNA sequence analyses showed that two of the cDNAs encoded the entire precursor polypeptides of Fd I and Fd III, which were composed of 150 and 152 amino acid residues, respectively, and the other encoded a 135 amino acid precursor polypeptide of Fd not yet identified. High degrees of homologies were found in the deduced amino acid sequences of mature regions of these Fd isoproteins, but the transit peptide of Fd III differed considerably from those of other Fd isoproteins. Fd I and the unidentified Fd were encoded mainly with codons ending in C or G, but such strong codon bias was not seen in Fd III. Gene specific probes for each cDNA were used to probe Northern blots of RNA isolated from leaves, mesocotyls, and roots of maize seedlings. The gene transcripts for Fd I and the unidentified Fd were restricted to leaves and their levels increased markedly upon illumination of etiolated seedlings, whereas that for Fd III was detected in all organs and its accumulation was not light dependent. This organ specific accumulation of Fd mRNAs corresponds exactly to the distribution pattern of Fd isoproteins.

Since higher plant Fd was first recognized as a component of the photosynthetic electron transport chain, Fds have been shown to play a central function in many light-dependent metabolic processes (for review, see ref. 1). They are localized in the stroma of chloroplasts where they serve to mediate electron flow from PSI to a variety of Fd-linked enzymes.

Recently, it was reported that nonphotosynthetic organs, such as maize roots and mesocotyls (12), and radish white roots (25) also contained Fd. These Fds are most probably involved in electron transfers from pyridine dinucleotides to some Fd-linked enzymes in a light-independent manner (23, 25), although subcellular location of the Fds and detailed metabolic processes are still unknown. The 'nonphotosynthetic' Fd is known to be distinct from the 'photosynthetic' Fd by immunological and chemical criteria (12, 26). In addition to the structural difference, the two types of Fd differ in the manner of expression. We have reported (12) that young maize seedlings contain at least four Fd isoproteins (Fd I to Fd IV) and that Fd I and Fd II are only found in leaves, whereas Fd III and Fd IV are distributed in all parts of the seedlings. Furthermore, the leaf-specific Fds are light inducible, whereas the others are not affected by light. It is likely that most plants have these two types of Fd, namely, leaf specific and nonspecialized Fds.

The polypeptide of leaf Fd is nuclear coded, synthesized as a larger precursor, and imported posttranslationally to the chloroplasts (9). In the chloroplast stroma, the precursor is processed to the mature size (20, 21) and a 2Fe-2S cofactor is incorporated into the mature polypeptide to produce a functional molecule (24). Three cDNAs for leaf Fds from *Silene pratensis* (21), spinach (27), and pea (3) have been cloned, and the structures of the precursor proteins with an NH_2[4]-terminal extension were reported. Southern blot analysis of genomic DNA using the cDNAs as a probe suggested that leaf Fd is coded by one or two genes (3, 20). Fd genes encoding pea Fd I *(Fed-1)* (5) and *Arabidopsis thaliana* Fd *(Fed A)* (22) have been cloned, and the promotor regions of these two genes contain plant promotor consensus sequences probably involved in expression of the leaf Fd gene. It was also proposed that light-induced accumulation of Fd mRNA in pea is not controlled by the 5' upstream of *Fed-1,* but the transcribed portion of *Fed-1* conveys a responsiveness for the light regulation (4).

These background data raise interesting questions regarding the possible differences between the leaf-specific Fd and the nonspecialized Fd: (a) what are the fundamental differenes in the structures and functions of the two-types of Fd; (b) what is the intracellular location of Fd in nonphotosynthetic organs and does it have a larger precursor; (c) how are the light-inducible and noninducible characteristics of the two types of Fd regulated? To answer these questions, we have analyzed the maize Fd gene family. In this initial study, we report on the molecular cloning and characterization of three groups of

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[4] Abbreviations: NH_2, amino; PVDF, polyvinylidene difluoride; COOH, carboxy; XXG/C codons, codons ending in G or C; bp, base pair.

cDNAs for maize Fds including both leaf-specific and non-specialized isoproteins.

MATERIALS AND METHODS

Plant Materials

Maize (*Zea mays* L. cv Golden Cross Bantam T51) seedlings were grown on vermiculite at 25 to 28°C in a dark room for 6 d. The resulting etiolated seedlings were then greened under a continuous light of fluorescent tubes with an intensity of about 300 $\mu E/m^2s$ for desired periods. Harvest of the etiolated seedlings was carried out under green safelights. To obtain a large amount of etiolated plants for isolation of the Fd isoproteins, maize seeds were germinated hydroponically in plastic trays for 4 to 6 d under a dim light. Mature green leaves were obtained from plants grown in a greenhouse for a few weeks under natural light conditions.

Purification of Fd Isoproteins

Etiolated seedlings were divided into shoots and roots, and stored at -20°C until required. About 3 kg of frozen shoots were homogenized in 3 l of an ice-cold extraction buffer (20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 40% [v/v] acetone) with a Waring blender. The homogenate was filtered through two layers of cheesecloth and centrifuged at 10,000*g* for 20 min, and the resulting supernatant was passed through an excess amount of DEAE-cellulose packed in a column (5.5 x 10 cm). After washing the column with Tris buffer (50 mM Tris-HCl, pH 7.5) containing 100 mM NaCl, the adsorbed proteins were eluted with Tris buffer containing 700 mM NaCl. The eluted proteins were fractionated by addition of ammonium sulfate to 70% saturation, and the precipitates were removed by centrifugation at 12,000*g* for 10 min. The Fd contained in the supernatant was directly concentrated using a small DEAE-cellulose column as described in (12) and chromatographed on a Sephadex G-75 column (1.8 x 60 cm), which was equilibrated and developed with Tris buffer containing 150 mM NaCl. Fractions containing Fd were combined and the four Fd isoproteins were separated by chromatography on a DEAE-Sephacel column (1.4 x 25 cm) using a linear gradient of NaCl (400 mL) from 150 to 500 mM in Tris-buffer. Fd was prepared from roots and green leaves by essentially the same procedures as above.

Antibodies

Antibodies were raised against Fd I or a mixture of the four Fd isoproteins in Japanese white rabbits by injection of 200 μg protein emulsified with complete Freund's adjuvant. One month after the initial injection, the rabbits were boosted three times at 1 week intervals with 100 μg of the antigens. The antibodies raised against Fd I reacted predominantly with Fd I and Fd II, and those raised against a mixture of the Fd isoproteins recognized essentially all the isoproteins. For immunoscreening of cDNAs coding for Fd polypeptides, antibodies were affinity purified with antigen bound to PVDF membrane according to the published procedure (11).

Analysis of Amino Acid Sequence and Composition

Fd isoproteins prepared from the etiolated seedlings were separated by nondenaturing PAGE (12) and electroblotted onto PVDF membrane and their NH_2-terminal sequences were directly determined with a gas-phase sequencer (Applied Biosystems, model 477 A) as described previously (14). Amino acid composition and COOH-terminal sequence of Fd I and Fd II purified from green leaves were determined in an amino acid analyzer (JEOL, model JLC-300) after acid hydrolysis and carboxypeptidase A digestion, respectively, as previously described (8).

Preparation of Poly(A)$^+$ mRNA

Total RNA was isolated by the guanidine thiocyanate procedure (15) from about 30 g of leaves of the seedlings which were illuminated for certain periods, after being grown for 6 d in the dark. Poly(A)$^+$ mRNA was isolated from total RNA by oligo(dT)-cellulose fractionation (19).

Construction and Screening of cDNA Library

Synthesis of cDNA from the poly(A)$^+$ RNA and construction of cDNA libraries in λ-gt11 or pUEX1 were carried out using a cDNA cloning kit (Amersham, cDNA synthesis system plus and cDNA cloning system λ-gt11 or plasmid pUEX1) essentially as described by the supplier. The libraries were screened using the affinity-purified antibodies. The antibodies bound to fusion proteins on nitrocellulose filters were visualized by the reaction with alkaline phosphatase conjugated goat anti-rabbit IgG.

A second screening was conducted to obtained longer cDNAs by nucleic acid hybridization techniques (17) using an insert DNA obtained from a positive clone isolated at the first immunological screening.

Subcloning and Sequence Analysis

Insert DNAs were excised from recombinant phage and plasmids by cutting them with *Eco*RI and *Bam*HI, respectively, and subcloned into pUC19. The subclones were mapped with various restriction endonucleases, and appropriate fragments were cloned into M13mp18 or mp19 for DNA sequence analysis. In some cases, a set of deletions was introduced to M13 clones according to the method of Yanisch-Perron *et al.* (28). DNA sequences were determined by the dideoxy chain termination method (18), and conventional DNA techniques described by Maniatis *et al.* (17).

Northern Blot Analysis

Total RNA was prepared separately from roots, mesocotyls, and leaves including coleoptiles of etiolated and greening seedlings. The RNA was denatured with formaldehyde and subjected to electrophoresis on a 1% agarose gel containing formaldehyde as previously described (17). The RNA was blotted onto nylon membrane (Hybond-N$^+$, Amersham) and probed with gel-purified subfragments of cDNA inserts. The probes were labeled by a random-primed method (6) in the presence of [^{32}P]dCTP. Prehybridization and hybridization of

the transferred filters were in 50 mM Na-phosphate buffer (pH 6.5), 5 × Denhardt's solution (1 × Denhardt's solution: 0.02% [w/v] Ficoll, 0.02% [w/v] PVP, 0.02% [w/v] BSA), 5 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M Na-citrate), 0.1% SDS, 250 μg/mL denatured salmon sperm DNA, and 50% formamide at 42°C. Filters were washed in 2 × SSC and 0.1% SDS at room temperature and then in 0.1 × SSC and 0.1% SDS at 50°C.

RESULTS

Separation and Structural Analysis of Fd Isoproteins

A mixture of the four Fd isoproteins prepared from etiolated seedlings was chromatographed on a DEAE-Sephacel column and the fractions containing each of the four isoproteins as a main component were combined (Fig. 1). The Fd isoproteins thus obtained were electrophoresed on a nondenaturing gel, electroblotted onto PVDF membranes, and subjected to sequence analysis. As shown in Figure 2, the first 20 NH_2-terminal residues of Fd I and Fd II were identical, whereas those of Fd III and Fd IV differed. The Fd isoproteins prepared from green leaves (Fd I and Fd II) and roots (Fd III) had the same NH_2-terminal structures as the corresponding molecular species from etiolated seedlings (data not shown). The analyses by carboxypeptidase A digestion revealed that Fd I and Fd II had different COOH-termini (Fig. 2). These results indicate that the four isoproteins are homologous, but distinct from one another, and thus coded by different genes.

Isolation and Characterization of cDNA Clones Coding for Fd Isoproteins

The amounts of Fd I and Fd II present in etiolated seedlings were increased by illumination (12), suggesting that the relative abundance of the messages for the isoproteins might vary significantly according to growth conditions. We have synthesized cDNA using two different batches of poly(A)$^+$ RNA prepared from the leaves of greening seedlings illuminated for 6 h or for 2 d. The cDNAs from the longer and shorter illuminated plants were ligated into λ-gt11 and pUEX1, respectively, to construct two different cDNA libraries.

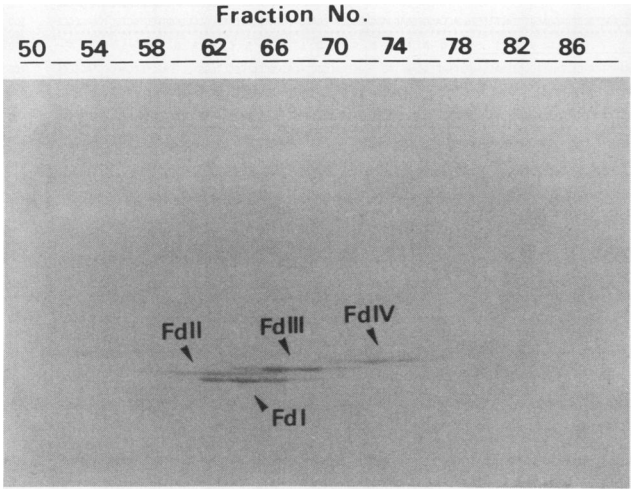

Figure 1. Separation of maize Fd isoproteins by DEAE-Sephacel column chromatography. A mixture of semipurified Fd isoproteins was chromatographed on a DEAE-Sephacel column under the conditions as described in "Materials and Methods." Fractions (4 mL) were collected and analyzed by nondenaturing PAGE to monitor separation of the isoproteins. This figure shows the gel stained with Coomassie brilliant blue. Fraction tubes, 60 to 62, 63 to 65, 66 to 68, and 69 to 72, contained Fd II, Fd I, Fd III, and Fd IV as a main component, respectively.

Amino-terminal sequence

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          1       5        10        15        20
Fd I    A-T-Y-N-V-K-L-I-T-P-E-G-E-V-E-L-Q-V-P-D-D-V-Y-I-
Fd II   A-T-Y-N-V-K-L-I-T-P-E-G-E-V-E-L-Q-V-P-D-
Fd III  A-V-Y-K-V-K-L-V-G-P-E-G-E-E-H-E-F-
Fd IV   A-V-Y-K-V-K-L-I-G-P-E-G-Q-E-H-V-L-
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Carboxy-terminal sequence

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Fd I   -(L,T,G)-A
Fd II          -L
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Figure 2. Terminal amino acid sequences of maize Fd isoproteins. The NH_2-terminal sequences were determined with a gas-phase sequencer. A carboxypeptidase A digestion of Fd I released free alanine (0.29, 0.63, and 0.98 mol/protein), glycine (0,14, 0.47, and 0.98), threonine (0.10, 0.44, 0.98), and leucine (0.13, 0.40, and 1.00) for 30, 60, 180 min incubations, respectively, and that of Fd II released only leucine for the same incubation times as above. The COOH-termini of Fd I and Fd II are thus determined to be alanine and leucine, respectively, and the order of glycine, threonine, and leucine following the COOH-terminus of Fd I is not determined on the basis of the digestion profile.

The λ-gt11 librarly was screened with an antibody raised against Fd I, and 4 plaques obtained from 2 × 10^5 recombinant phages gave a strong immuno-reactivity during several rounds of screening. After subcloning *Eco*RI inserts into the vector pUC19, the resulting plasmids designated as pFD1, pFD1-1, pFD1′-1, and pFD5 with 742, 377, 328, and 627 bp *Eco*RI fragments, respectively, were further analyzed. Sequence studies (Figs. 3 and 4) showed that the inserts of pFD1 and pFD5 contained open reading frames encoding polypeptides composed of 150 and 135 amino acids, respectively. The other clones, pFD1-1 and pFD1′-1 had partial coding sequences lacking the NH_2-terminal region; the sequence of pFD1–1 was identical to the corresponding region of pFD1 and that of pFD1′-1 was very similar to, but distinct from pFD1 (data not shown). The same cDNA library was then rescreened by nucleic acid hybridization with the insert of pFD1 as a probe under low stringency conditions. Sixteen hybridizing plaques were isolated. Each clone was found to belong to one of the three groups of cDNA; one clone to pFD5, nine clones to pFD1, and six clones to pFD1′-1. The longest cDNA among the third group, designated as pFD1′, was sequenced (Figs. 3 and 4). The DNA insert of pFD1′ was composed of 746 bp and contained the coding sequence for a polypeptide of 150 amino acids identical with that of pFD1. The two clones differ only at two nucleotide sites in the coding regions and have about 80% homology in the noncoding regions. This was probably due to an allelic variation of our maize strain used for the experiment as described in the "Discussion."

The amino acid sequences deduced from the nucleotide

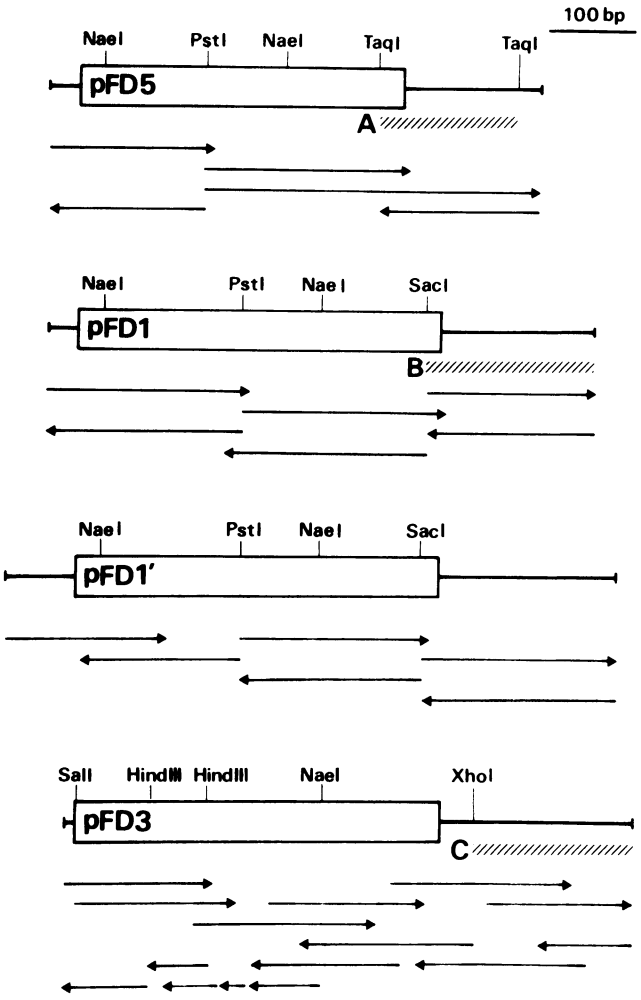

Figure 3. Restriction maps and sequence strategies of four cDNAs encoding maize Fd isoproteins. Relevant restriction sites are indicated to distinguish four clones, pFD5, pFDI, pFD1′, and pFD3. Fragments obtained by digestions with various restriction enzymes were subcloned into M13 vectors, and their nucleotide sequences were determined. Arrows indicate the direction and extent of sequences determined. The coding region is represented by an open bar. Probes A, B, and C used for further studies are obtained from hatched regions of pFD1 (*TaqI* fragment), pFD2 (*SacI*/*Eco*RI fragment), and pFD104 (*XhoI*/*KpnI* fragment), respectively.

sequences of the cloned cDNAs were compared with the terminal structures of the Fd isoproteins. The NH_2-terminal sequences of Fd I and Fd II, which were identical up to 20 residues (Fig. 2), were found both in pFD1(pFD1′) and pFD5 beginning from Ala53 and Ala39, respectively. The COOH-terminal structure of the polypeptide coded by pFD1(pFD1′), -Leu-Thr-Gly-Ala, matched with that of Fd I, indicating that pFD1(pFD1′) coded for a precursor polypeptide of Fd I. This was also confirmed by a comparison of the amino acid composition of Fd I and that of a mature polypeptide coded by pFD1(pFD1′) (data not shown). The COOH-terminal sequence of the polypeptide coded by pFD5 did not agree with that of Fd II. At present, the product of pFD5 has not been identified.

The other cDNA library constructed in pUEX1 was screened with an antibody raised against a mixture of the four Fd isoproteins. Three confirmed positive clones were obtained from 2×10^5 recombinant colonies. Two of them were subsequently found to be the same clone as pFD1 and pFD5, and the third clone designated as pFD3 was a new clone distinct from any of pFD1, pFD1′, and pFD5. Sequence analysis showed that pFD3 contained an open reading frame of 456 bp coding for a polypeptide of 152 amino acid residues (Figs. 3 and 4). The deduced sequence beginning from Ala56 matched exactly with the NH_2-terminal sequence of Fd III, which was unique among the four Fd isoproteins.

Differential Distribution of Fd mRNAs in Etiolated Seedlings

To study the independent expression of the Fd isoprotein genes, restriction fragments from the 3′ ends of the cDNAs (Fig. 3) were used as probes which would not cross-hybridize

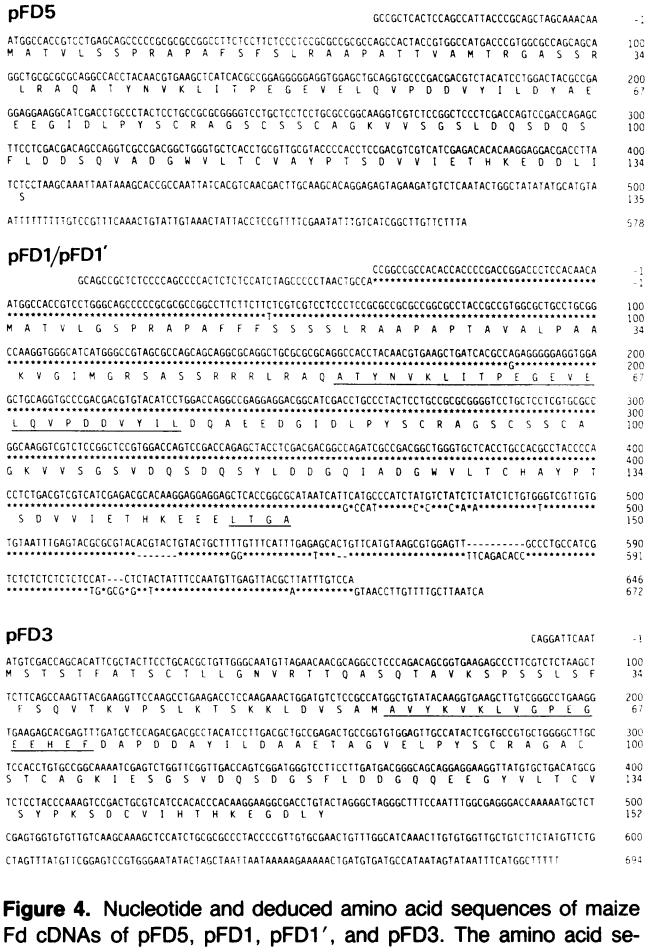

Figure 4. Nucleotide and deduced amino acid sequences of maize Fd cDNAs of pFD5, pFD1, pFD1′, and pFD3. The amino acid sequence coded by an open reading frame is shown below the nucleotide sequence and the region which has been found in the determined protein sequence is underlined. The nucleotide sequence of pFD1′ is shown below that of pFD1; only different bases are indicated and identical bases are marked with an asterisk. Gaps are inserted to obtain the best homology. Note that the deduced amino acid sequences of pFD1 and pFD1′ are identical.

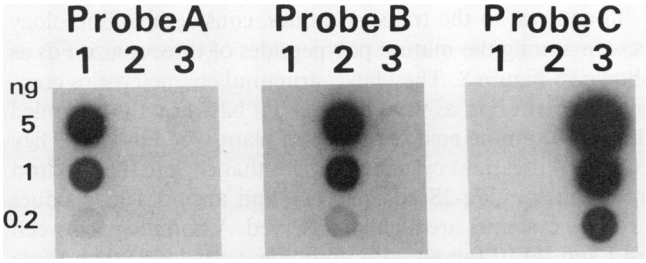

Figure 5. Specificity of probes for three different cDNAs. Probes A, B, and C were excised and gel-purified from the 3'-side of pFD5, pFD1, and pFD3, respectively, as shown in Figure 3. The original cDNA (5, 1, and 0.2 ng) was spotted on the nylon membrane and hybridized with the ^{32}P-labeled probes: lane 1, pFD5; lane 2, pFD1; lane 3, pFD3.

with mRNA from the different genes. When these probes were hybridized with each of the total cDNA fragments, little or no cross-hybridization was observed for the heterologous cDNAs under conditions in which strong signals were obtained for the homologous cDNA blots (Fig. 5).

Total RNA was isolated separately from roots, mesocotyls, and leaves of etiolated seedlings. A Northern blot of the total RNAs was separately probed with the specific probes. As shown in Figure 6, the probes for pFD5 and pFD1 hybridized only with leaf RNA around 700 and 800 nucleotides, respectively, whereas the probe for pFD3 hybridized with the RNAs from all organs. The transcript for pFD3(Fd III) was larger by about 200 nucleotides than those for pFD1(Fd I) and pFD5. This distinct difference of the distribution between mRNAs for pFD1(Fd I) and pFD3(Fd III) coincided with the previous observation on the isoprotein levels in each organ (12).

Light-Induced Accumulation of Fd mRNAs in Leaves

The kinetics for change of mRNA levels for the three cDNA were determined during the greening of etiolated seedlings. Figure 7 shows total RNA blot analysis of the Fd mRNAs as a function of greening time. The steady-state level of the mRNAs for pFD1(Fd I) and pFD5 began to increase within the first 12 h of illumination and reached at least 10-fold over the duration of the experiment. The increase of Fd I transcript seemed to be slightly more rapid than the other. In the seedlings kept in the dark, no increase was observed. These results indicate that light induction of Fd I at protein level can be attributed to the accumulation of the transcript for this isoprotein. The transcript level of pFD3(Fd III) was essentially not influenced by light.

DISCUSSION

Four cDNA clones, pFD1, pFD1′, pFD3, and pFD5, encoding maize Fd isoproteins have been isolated and characterized. By comparing the polypeptide structures of the four Fd isoproteins (Fd I to Fd IV) with the deduced amino acid sequences of the cDNAs, pFD1 (pFD1′) and pFD3 were identified to encode Fd I and Fd III, respectively. The polypeptide encoded by pFD5 did not match with any of the four isoproteins, although the transcript for this cDNA was apparently present in leaves. This implies that a molecular species other than the four Fd isoproteins would be present in maize seedlings at a very low concentration. In this study, cDNAs for Fd II and Fd IV were not obtained, although the protein levels of these Fds are comparable to Fd I and Fd III in etiolated seedlings (Fig. 1). It is unlikely that Fd II and Fd IV are modified forms of Fd I and Fd III, respectively, because definite structural differences are found among them (Fig. 2). At present, we have no explanation for missing the cDNAs.

The existence of pFD1 and pFD1′, which encode the identical polypeptide corresponding to Fd I, seems to be attributed to an allelic variations according to the following reasons (Y Kimata, T Hase, unpublished results): (a) the maize cultivar used for this study was a hybrid of two parental inbreds, P51B and P39, both of which contain the same compositions of Fd isoproteins as the hybrid; (b) a Southern blot analysis of genomic DNA of the hybrid showed that the genome gave two restriction fragments hybridized with the specifc probe for pFD1 (probe B in Fig. 3), and that one of them was deprobed more easily than the other by washing with increasing stringency, suggesting that the strongly and weakly hybridizing fragments corresponded to pFD1 and pFD1′, respectively; and (c) the same genomic analysis of the inbreds showed that both inbred lines gave only one hybridizable band, and that the strengths of the hybridization in P51B and P39 corresponded to those for pFD1 and pFD1′, respectively. Therefore, we presume that pFD1 and pFD1′

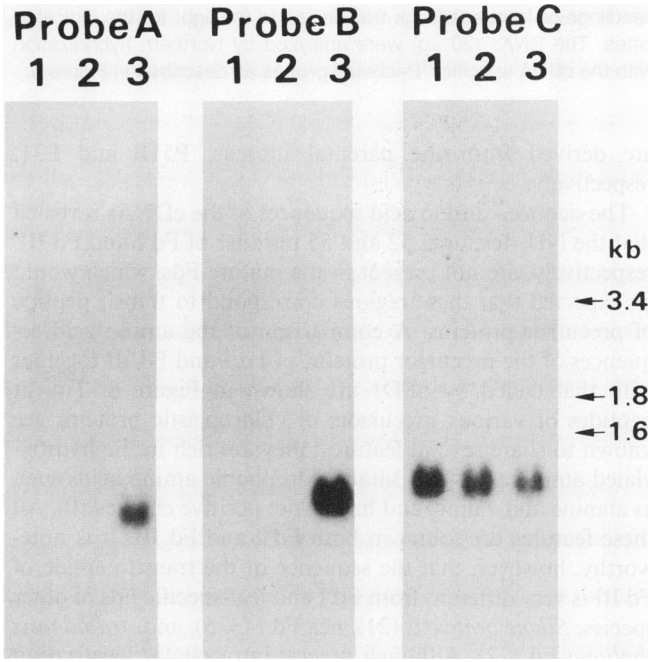

Figure 6. Northern analysis of mRNAs for Fd isoproteins in different organs of maize seedlings. Total RNAs (16 μg) from roots (lane 1), mesocotyls (lane 2), and leaves (lane 3) of seedlings were electrophoresed and transferred to nylon membrane. Three comparable blots derived from the same gel were separately probed with the cDNA specific ^{32}P-labeled probes: probe A, pFD5; probe B, pFD1; probe C, pFD3. The migration of ribosomal RNAs are indicated at the right of the figure.

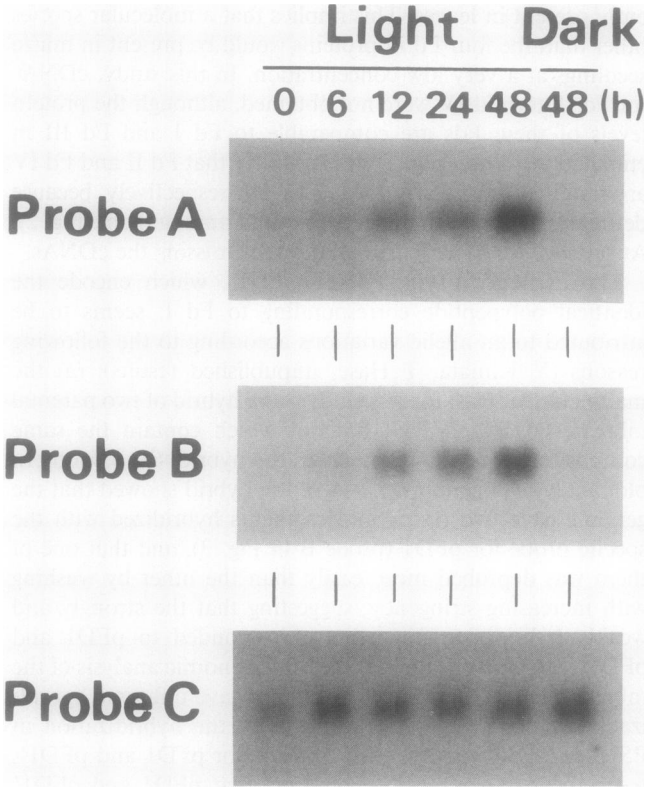

Figure 7. Effect of light on the accumulation of mRNAs for Fd isoproteins. Leaf total RNA was isolated from 6-d-old, dark-grown seedlings or from seedlings transferred to the light for the indicated times. The RNAs (20 μg) were analyzed by Northern hybridization with the cDNA specific ^{32}P-labeled probes as described in Figure 6.

are derived from the parental inbreds, P51B and P31, respectively.

The deduced amino acid sequences of the cDNAs revealed that the NH_2-terminal 52 and 55 residues of Fd I and Fd III, respectively, are not present in the mature Fds, which would be expected that these regions correspond to transit peptide of precursor proteins. A comparison of the amino acid sequences of the precursor proteins of Fd I and Fd III together with that coded by pFD1 are shown in Figure 8. Transit peptides of various precursors of chloroplastic proteins are known to share several features; they are rich in the hydroxylated amino acids and small hydrophobic amino acids such as alanine and valine, and have a net positive charge (10). All these features are found in both Fd I and Fd III. It is noteworthy, however, that the sequence of the transit peptide of Fd III is very different from Fd I and leaf-specific Fds of other species, *Silene pratensis* (21), pea Fd I (3, 5), and *Arabidopsis thaliana* Fd (22). Although precise intracellular localization of Fd III is not yet determined, the transit peptide of Fd III probably functions as an import signal to plastids. An *in vitro* synthesized precursor for Fd III as well as that for Fd I is imported into isolated chloroplasts (S Suzuki, T Hase, unpublished result). Our preliminary experiment on subcellular fractionation of root and mesocotyl protoplasts showed that Fd III was associated with a particulate fraction rich with plastids.

In contrast to the transit peptides, considerable homology is seen among the mature polypeptides of three maize Fds as shown in Figure 8. They have structural characteristics common to plant Fds, as were pointed out based on the compiled data of the amino acid sequences of plant-type Fds (13). They contain 4 invariant cysteine residues that chelate the two iron atoms in the 2Fe-2S cofactor (7), and amino acid residues near the cysteines are highly conserved. A homology between Fd I and Fd III (about 64% homology) was lower than those among leaf-specific Fds from maize and other plant species (70–80% homology). This lower level of homology between Fds existing in different organs has also been reported for radish Fd isoproteins (26). In the comparison between the two plant species, it is remarkable that the similarities between maize Fd I and raddish leaf Fd (76% homology) and between maize Fd III and radish root Fd (78%) are higher than those obtained from the other combinations (64–67%). This may indicate that a gene duplication which resulted in an appearance of the two types of Fd preceded plant speciation. It is still an open question as to whether the two types of Fd are functionally different.

The cloning of the cDNAs for the maize Fd isoproteins led to analyses of their mRNA levels in different organs and under different physiological conditions. Northern hybridization using gene specific probes (Fig. 6) shows that the organ specific accumulation of Fd mRNAs reflects exactly the distribution pattern of the Fd isoproteins (12). This correlation suggests that much of the regulation on the organ specific expression of Fd genes may be at under transcriptional level, although participation of a posttranscriptional event such as differential stability of the transcripts depending on organs cannot be ruled out.

The expression of the leaf Fd gene has been shown to be under phytochrome control in pea (3). Furthermore, a recent report suggests that light-regulated accumulation of the pea leaf Fd mRNA is not controlled by 5' upstream sequences of the Fd gene, but instead, the transcribed portion contains light regulatory elements, which may involve alterations of the transcript stability (4). Our present data show the tran-

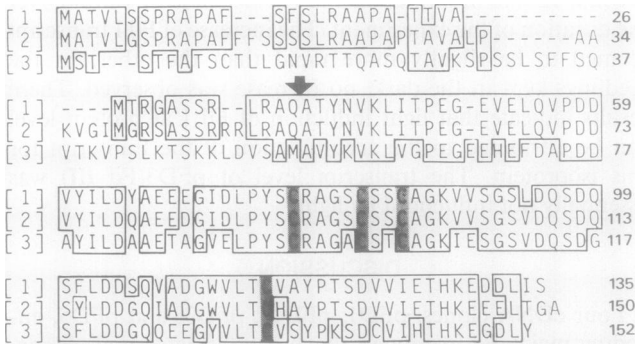

Figure 8. Comparison of the amino acid sequences of the maize Fd isoproteins. The amino acid sequences deduced from pFD5 [1], pFD1 [2], and pFD3 [3] are aligned. Gaps are inserted to obtain the best homology. Identical amino acid residues are boxed, and the four conserved cysteine residues necessary for ligation of 2Fe-2S cofacter (7) are shadowed. An arrow indicates the processing site of precursor.

script level of the leaf-specific Fd increases rapidly upon illumination of maize seedlings, whereas that of the nonspecialized Fd remains almost constant (Fig. 7). This clear-cut difference between the two types of Fd gene presents a unique opportunity to examine the organ specific expression and light regulation of Fd genes.

Interestingly, a strong codon bias is found in maize leaf-specific Fd cDNAs in contrast to the nonspecialized Fd cDNA; pFD1 and pFD5 exclusively use codons ending in G or C (95 and 96% XXG/C codons, respectively) and pFD3 shows no such bias (67% XXG/C codons). A similar observation has been made for the three genes encoding the maize catalase isoenzymes, and tissue-specific expression appears to be related to the codon usage (16). Such codon bias is, however, restricted mainly in monocots (2). Leaf Fd genes of spinach (27), pea (3), *S. pratensis* (21) have no codon bias toward to XXG/C codons as generally observed in dicot genes (2). At present, a simple structural comparison of Fd cDNAs from maize and other plant sources is unable to reveal consistent similarity and/or difference between the two types of Fd, even if light regulatory elements reside within transcribed sequences. Further studies including comparative characterization of genomic DNA of the two types of the Fd gene will be necessary to examine the organ-specific and light-regulated expression of Fd. Work is currently in progress on the cloning of the leaf-specific and nonspecialized Fd genes.

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